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SERUM DIAGNOSIS OF INFECTIOUS EQUINE ENCEPHALOMYELITIS

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[On the specific example of infectious equine encephalomyelitis, this article describes a rather novel method of diagnosing virus diseases. This method is being applied in the USSR in both human and veterinary medicine.]

During 1946 - 1949, we studied methods of specific serum diagnosis of equine virus encephalitis, with particular attention to RSK (reaction of complement fixation) and VBA (virus-bacterial agglutination) reactions.

At present, we have data on these reactions. These data were obtained by experimenting on rabbits as well as by carrying out field tests. By experimenting with rabbits, we established the specificity of RSK and VBA as reactions for the diagnosis of experimental equine encephalomyelitis. We further established that, in both hyperimmunization of rabbits and intracerebral infection of horses with the virus, complement-fixing and agglutinating antibodies appear in peripheral blood within a very short time after introduction of the virus antigen into the animal (in 48-72 hr after intracerebral, intravenous, or intraperitoneal introduction of the virus.) After obtaining encouraging results on rabbits, we attempted to evaluate both reactions in regard to their usefulness for diagnosing early as well as old cases of equine encephalomyelitis.

Before discussing the results of our observations on horses, we will outline the technique of the reactions. In order to carry out virus-bacterial agglutination, the antigen in question and horse serum (which may be inactivated) are needed. The antigen for the agglutination reaction with equine encephalomyelitis virus was prepared in the following manner. An emulsion in physiological solution of fresh virus which had been passed through rabbits was

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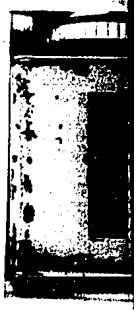

filtered through a Seitz filter and loaded onto a 2-day-old culture of B. prodigiosum which had been appropriately standardized. The loading was so perfect that repeated washing on the centrifuge did not remove the virus from the bacteria.

The VBA reaction was carried out by the test-tube method. Serum of horses which had the disease and which were in contact with diseased animals as well as serum of horses in the preclinical stage of encephalomyelitis gave a positive agglutination reaction for the homologous virus in appropriate dilutions. Sera which gave a positive reaction with the antigen as prepared above did not agglutinate with the virus alone or a culture of B. prodigiosum not loaded with specific virus.

On the other hand, sera of horses which exhibited a positive reaction to the specific antigen did not produce the least trace of agglutination in B. prodigiosum loaded with filtered suspension of the brain of normal animals, just as they did not in B. prodigiosum alone.

The reaction was carried out as follows. After the antigen has been added to the diluted serum, the test tubes were shaken and kept for 2 hr at 42° on a water bath and then for 24 hr at room temperature. The results of the agglutination were estimated twice: once after removing the samples from the water bath and once on the next day.

As far as the reaction of complement fixation is concerned, we found that the usual method of setting it up in such a manner that both phases are carried out on a water bath or in a constant temperature closet gave very scattered and unconvincing results. On the other hand, carrying out the reaction in the cold, we invariably got satisfactory results. The brain antigen we prepared (from fresh brain of rabbit through which the disease had been passed) was active for over a month when stored at +4° to +6°.

This antigen was used in the RSK reaction in a quantity of 0.2 ml. Three quarters of a working unit of complement, a triple dose of hemolysin as compared with the working unit and a 3% suspension of ram erythrocytes in a strictly isotonic solution of chemically pure sodium chloride were added. The sera were first diluted 1:5 and in this dilution inactivated at 65° during 20 min. In the main test, each serum was diluted from 1:10 up to 1:800 and higher (the same dilutions were made for VBA.)

Both reactions were always carried out on the same day. The serum of all horses which had been tested was investigated by both reactions. The two reactions were applied to 298 horses. A group of 186 horses was used for control purposes. Among the horses investigated, there were, during the past 4 years, some cases of infectious encephalomyelitis. Out of 299 heads, nine had had the disease, exhibiting its typical clinical aspects. The rest of the horses were in more or less prolonged contact with animals which either died of the disease or had the disease and recovered. By using both serum tests (and applying them repeatedly,) we found that 164 horses (55%) showed a negative reaction to both of them, that 89 horses (29.8%) showed sharp positive reactions, and that 46 horses (15.2%) showed a doubtful reaction.

Entirely different results were obtained in the investigation of control horses which had had no diseases with neurological symptoms between 1944 and 1949. Out of 186 control animals investigated by the use of both tests, not a single one exhibited a positive reaction. The sera of 22 horses (11.8%) showed doubtful reactions.

In the course of experiments on rabbits, and then on horses, we found out that a positive VBA reaction must be assumed when an unbrella-shaped precipitation takes place with a serum dilution of 1:50 and higher. A positive titer for RSK is represented by a serum dilution of 1:25 or higher. When sera gave a positive VBA reaction in a dilution lower than 1:50 and a positive RSK reaction in a dilution lower than 1:25, the results were considered doubtful.

In experiments carried out on rabbits, virus-bacterial agglutination and the appearance of complement-fixing antibodies in the blood coincided and were dynamically similar. Parallelism of the two reactions (VBA and RSK) was also observed in horses, i.e., both reactions were either positive, doubtful, or negative. We did not find a single case where one of the reactions was positive and the other negative. The differences were only in titers. In the majority of cases, the VBA titer was higher than the RSK titer.

The results obtained on horses led us to the conclusion that the serodiagnostic reactions on which we are working must be considered specific. The same applies to data on rabbits.

The high percentage of positive reactions in the group of horses which had been exposed to encephalomyelitis indicated that encephalomyelitic virus infection of various clinical types is very common among horses. It is quite possible that, in addition to horses which exhibited noticeable clinical symptoms, a considerable number of horses had the disease but did not exhibit any symptoms. Both types of infected horses may have become virus carriers for long periods of time. Such virus carriers develop a humoral immunity, which is expressed clearly and sharply in positive immunity reactions. In this connection, one of Bosh'yan's conceptions is in agreement with considerable experimental material in our possession.

On the other hand, the group of control horses which had not been exposed to the infection showed negative reactions to both tests (with the exception of a few horses which gave a doubtful reaction.) These results show that the RSK and VBA reactions, as applied by us, are strictly specific and can be used in combating equine encephalomyelitis.

In 1948, we investigated a small focus of infection in which there were sick horses and, in close contact with them, healthy horses. On investigating ten horses, we found that animals which had been in contact with sick horses but did not give positive serodiagnostic reactions soon developed them as far as both RSK and VBA were concerned. Repeated investigation of the sera yielded the following results: an increase of titers in both reactions was shown for those horses which were in contact with sick animals, gave positive serological reactions, got the disease, and then recovered. The sera of two horses that died yielded high RSK and VBA titers at first, but these titers dropped as the day of death approached and were considerably lower on the day of death.

Apparently the reactions in question can be used for early, preclinical diagnosis as well as for establishing that certain animals have become virus carriers as a result of recent contacts. Definite conclusions on this will be possible as soon as more factual material has been accumulated.

The results on horses are in complete agreement with experimental data obtained on rabbits. These data show that humoral antibodies already appear in the early stages of the infection, when neurological symptoms have not yet appeared and any other symptoms of the disease are absent.

The antigens for both VBA and RSK can be prepared without much trouble and have a considerable stability. In view of the fact that the VBA reaction is just as specific as RSK, it apparently may be used alone.

Taking into consideration the simple technique of the VBA reaction and the length of time during which the virus-bacterial antigen remains active, we come to the conclusion that this reaction is suitable for application in remote localities equipped with small laboratories in which the agglutination reaction is carried out daily. Antigen for the VBA reaction ought to be produced atone of the central scientific research institutes or else at a biologicals plant.

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